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MA. Dubick, GM. Zaucha, DW. Korte, Jr., CE. Wade

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HSD is currently being evaluated in our laboratory as a resuscitation solution for the treatment of hypovolemia at a dose of 4 ml/kg body weight. Since a few reports of dextran toxicity, particularly of the kidney, have been cited in the literature, the present study evaluated the acute and subacute toxicity of HSD administered IV to beagle dogs. In the acute toxicity studies, animals were infused with a single dose of HSD or its components hypertonic saline (HS) or dextran (D-70), at the maximum tolerated dose (MTD; 20 ml/kg). Controls received Ringers lactate (RL). In the HSD-infused dogs, transient, but significant increases in serum ala aminotransferase (AT), asp AT and alkaline phosphatase (AP) were observed for the first 72 h. In most cases this increase was also observed in the HS group. In the subacute studies, dogs were infused daily with the MTD of the above test solutions. Serum ala AT activity was 2-3-fold higher in the HSD than the RL group for the first 3 days.

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Again a similar effect was observed in the HS group. Slight, transient increases in asp AT and AP activity were also observed in the HSD group. Higher LDH activity was only observed at day 14 in dogs infused with the MTD of HSD or HS. In both studies, no adverse effects on BUN or serum creatinine were observed and other transient changes in serum parameters were attributable to hemodilution induced by HSD. No gross or microscopic lesions were observed in any major organ. Considering the proposed therapeutic dose of HSD is only 4 ml/kg, it appears that its use should be associated with minimal or no adverse effects.

ACUTE AND SUBACUTE TOXICITY OF 7.5% HYPERTONIC SALINE/6% DEXTRAN-70 (HSD) IN DOGS

2. BIOCHEMICAL AND BEHAVIORAL RESPONSES.

Michael A. Dubick†, Gary M. Zaucha, Don W. Korte, Jr. and Charles E. Wade

Division of Military Trauma Research

Letterman Army Institute of Research

Presidio of San Francisco, CA 94129-6800

(415) 561-5816

†To whom correspondence should be addressed

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The opinions and assertions contained here in are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army nor the Department of Defense. (AR 360-5)

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on recision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

ABSTRACT

HSD is currently being evaluated in our laboratory as a resuscitation solution for the treatment of hypovolemia at a dose of 4 ml/kg body weight. A few reports of dextran toxicity, particularly of the kidney, have been cited in the literature, so the present study evaluated the acute and subacute toxicity of HSD administered IV to beagle dogs. In the acute toxicity studies, animals were infused with a single dose of HSD or its components of hypertonic saline (HS) or dextran (D-70), at the maximum tolerated dose (MTD; 20 ml/kg). Controls received Ringers lactate (RL). In the HSD-infused dogs, transient, but significant increases in serum ala aminotransferase (AT), asp AT and alkaline phosphatase (AP) were observed for the first 72 h. In most cases this increase was also observed in the HS group. In the subacute studies, dogs were infused daily with the MTD of the above test solutions. Serum ala AT activity was 2-3-fold higher in the HSD than the RL group for the first 3 days. A similar effect was observed in the HS group. Slight, transient increases in asp AT and AP activity were also observed in the HSD group. Higher LDH activity was only observed at day 14 in dogs infused with the MTD of HSD or HS. In both studies, no adverse effects on BUN or serum creatinine were observed and other transient changes in serum parameters were attributable to hemodilution induced by HSD. No gross or microscopic lesions were observed in any major organ. Considering the proposed

therapeutic dose of HSD is only 4 ml/kg, it appears that its use should be associated with minimal or no adverse effects.

Key words: Hypertonic resuscitation, Dextran-70, hypertonic saline, aminotransferases, creatinine, lactate dehydrogenase, dogs

INTRODUCTION

Over the past decade studies have shown that hypertonic crystalloid solutions, alone or in combination with dextran, can be an effective small volume resuscitation solution for the treatment of hypovolemic states.[1-3] Consequently, the introduction of HSD (7.5% NaCl/6% Dextran-70) has generated a number of experimental studies investigating its safety and efficacy, [4-6] and a new drug application for HSD is currently under review by the U.S. Food and Drug Administration.

Before HSD can be incorporated into a therapeutic regime, concerns over the potential toxicity of the dextran and hypertonic saline components of HSD must be addressed. In the nearly 50 years that dextrans have been employed as plasma volume expanders, few reports of their general toxicity have appeared in the literature. Associated side effects of dextran use have been attributed mostly to allergic reactions [7-11] or disturbances in hemostasis. [7,12-16] In general these effects have been attributed to doses exceeding 1 liter of high molecular weight dextrans (>100,000). On the other hand, reports of dextran-induced renal toxicity have been associated with repeated administration of high doses of dextran with an average molecular size distribution of 40,000. [17,18] Studies in rabbits have shown that repeated administration of large doses of Dextran-70 resulted in dextran accumulation in white blood cells, liver and spleen, yet no evidence for dextran-induced tissue damage has been observed [19,20]. Acute toxicity studies with dextrans at repeated doses of 10 to

80 ml/kg in mice, dogs and rabbits have shown circulatory overload as the primary adverse effect and overall toxicity of IV administered dextran was extremely low (G. Jonsson, personal communication).[21]

Use of hypertonic salt solutions have primarily raised concern over the potential neurological effects induced by a hypernatremia. Thus, as part of the new drug application to FDA for HSD, the present studies investigated the acute and subacute toxicity of HSD and its individual components in dogs. Particular attention was focused on evidence for renal or hepatic toxicity, as well as behavioral changes.

EXPERIMENTAL

Animals and Treatment

Separate acute and subacute toxicity studies were conducted under GLP conditions. In the acute studies, male (n=8) and female (n=8) beagles dogs, weighing initially 7.5 to 10.9 kg, were obtained from Ridglan Farms, Inc. (Mt Horeb, WI). They were individually housed in stainless steel runs and fed Purina Canine Diet 5007 and purified water, ad libitum. For the daily dosing subacute toxicity studies 60 beagle dogs (30 male, 30 female), weighing 8.7-14.4 kg were obtained from Hazelton-LRE (Kalamazoo, MI). Details pertaining to animal housing and husbandry have been previously described. [22,23]

In the single dose acute toxicity studies, dogs were infused IV via the cephalic or saphenous vein with the maximum tolerated dose (MTD) of 20 ml/kg of 7.5% NaCl/6% Dextran-70 (HSD), 6% Dextran-70 (D-70), 7.5% NaCl (HS) or Ringer's Lactate (RL) (Pharmacia AB, Uppsala, Sweden). RL was used as the control solution. In the studies in which dogs received daily infusions with doses of 12, 16, and 20 ml/kg, solutions were administered IV over a 5 min period.

In dogs infused with the single dose of HSD or D-70, blood samples were withdrawn prior to and 0.25, 1,2,3,7 and 14 days following infusion. Blood samples in the multiple dosing studies were withdrawn prior to dosing (baseline) and on days 1,2,3,7 and 14 prior to the infusions. Serum was separated from blood cells by centrifugation and stored at -20°C until analyzed.

Observations and Serum Chemistry

During the 14-day observation period, clinical observations were accomplished daily before dosing, 1 hour after dosing, and in the afternoon. All observations were performed by the same technician to minimize interpersonnel variation in the detection of behavioral disturbances. All observations complied with Letterman Army Institute of Research Standard Operating Procedures defining clinical observations and their documentation in toxicology studies.

Serum enzyme determinations, BUN, creatinine and assays for sodium, potassium and chloride were performed by the Analytical Chemistry Branch, Letterman Army Institute of Research. Commercial assays for alanine and aspartate aminotransferases (ala AT; asp AT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), creatine kinase (CK) and gamma glutamyl transpeptidase (GGTP), BUN, creatinine and chloride were adapted for use on a Cobas Fara II centrifugal fast analyzer (Roche Analytical Instruments, Belleville, NJ). Serum sodium and potassium concentrations were determined with a flame photometer (Instrumentation Laboratory, Lexington, MA).

Statistical Analysis

Data were analyzed by repeated measures ANOVA with treatment and time as the independent variables. Differences among groups

were further analyzed by Newman-Keuls multiple range test.[24] A $p<0.05$ was considered statistically significant.

RESULTS

Acute Studies

Clinical Observations

In the acute dog studies, clinical signs observed were grouped into behavioral and gastrointestinal categories (Table 1). With the exception of two cases of diarrhea and one case of tremors, all clinical signs were observed on Day 0 immediately following dosing and resolved to normal within 24 hours. Dogs receiving HSD or HS exhibited the greatest incidence of signs. No clinical signs were observed in animals receiving RL.

Behavioral signs were the most frequently observed category. Inactivity (9 of 16 dogs) was observed in all animals receiving HSD and HS, but only in one animal receiving D-70. By 4 hour after dosing, one HSD and one HS-treated animal remained inactive. At the next observation period, 24 hours after dosing, all had returned to normal activity levels. Disorientation was observed in dogs receiving HSD (4 animals) and D-70 (2 animals). Disorientation resolved by 2 hours in both D-70 and HSD-treated animals. At 24 hours after dosing no disorientation was observed. Tremors (6 of 16) were observed in the HS (4 animals) and HSD (2 animals) groups. One animal receiving HS exhibited tremors on Day 1, but the animal returned to normal by Day 2. Ataxia was observed on Day 0 in one animal receiving HS.

Gastrointestinal signs observed included vomiting (7 of 16 animals), excessive thirst (2 of 16), increased salivation (6 of 16), and diarrhea (2 of 16) (Table 1). Vomiting, excessive

thirst and increased salivation were observed only in animals receiving HSD and HS. Diarrhea was observed in one animal receiving HS (Days 12-13) and one animal receiving D-70 (Days 5-6). Diarrhea was not related to the time of dosing.

Serum chemistry

In dogs infused with a single acute dose of HSD or HS at the MTD, serum Na and K concentrations at 0.25 d following infusion were significantly higher and lower, respectively, in comparison to the other group- (Table 2). At the other times, Na and K groups concentration were similar among the groups throughout the experimental period.

In dogs infused with a single dose of HSD at the MTD, a transient rise in serum ala and asp aminotransferase (AT) and alkaline phosphate was observed for the first 2-3 days following infusion (Figs 1&2). No differences in LDH activity were observed following HSD infusion or its individual components (Fig 2). Ala AT was also elevated in serum from HS-infused dogs for the first 2 days following infusion, while AP activity was elevated in serum for up to 3 days following D-70 infusion (Fig 1&2). CK and GGTP activities in serum were not significantly affected by infusion of HSD or it components (data not shown).

In these animals, BUN was significantly lower than baseline values 0.25 day following HSD infusion, while serum creatinine concentrations were lower at this time in both the HSD and HS group (Table 3). BUN and creatinine were not significaty different among the groups at the other time points (Table 3).

Subacute Studies

Clinical observations

In the subacute studies, the clinical signs observed were also grouped into behavioral, gastrointestinal categories (Table 4). An "Other" and a "General" category was also included and primarily represented respiratory disturbances. With the exception of soft stool, which exhibited an equivalent incidence among all groups, all major clinical signs were observed with greatest incidence in dogs receiving HSD or HS. The incidence of each individual sign was approximately the same among the HSD- or HS- treated groups. The incidence of major signs in D-70 treated groups was intermediate between the HSD- or HS-treated groups, and those treated with RL. Signs occurred only sporadically in RL-treated dogs. With the exceptions of an increased incidence of excessive salivation in the middle and high-dose groups receiving HSD or HS, and an increased incidence of tremors in the middle and high-dose groups receiving HS or D-70, the incidence of signs was not dose-related. No sex-related difference were apparent in any of the clinical observations.

Behavioral disturbances were also the most frequent clinical observations in the subacute studies (Table 4). Behavioral signs observed included disorientation (48 of 60 animals), inactivity (47 of 60), tremors (40 of 60), hyperactivity (6 of 60), pacing (5 of 60), circling (3 of 60) and staggering (2 of 60). Disorientation, inactivity and tremors were observed with

greatest incidence in HSD- and HS-treated animals. A moderate incidence of these three signs was observed in D-70-treated animals, while the signs appeared only sporadically among those receiving RL. The incidence and severity of behavioral signs were greatest one hour after dosing each day. A gradual reduction in incidence and severity then occurred until most signs resolved within 24 hours after dosing. The signs reappeared after the next day's dosing, and repeated the cycle of resolution over the following 24 hours. Hyperactivity, pacing, circling, and staggering occurred sporadically throughout the study period, and were randomly distributed among the groups.

General signs observed included excessive thirst (32 of 60), hunched posture (31 of 60), increased salivation (29 of 60), decreased appetite (15 of 60), excessive bleeding from the injection site (2 of 60), swelling or edema of the injected leg (2 of 60), and bloody urine (1 of 60) (Table 4). Excessive thirst, hunched posture, and increased salivation were observed primarily in animals receiving HSD or HS. Increased salivation was the only sign that appeared dose related, and was observed primarily in the middle and high-dose groups treated with HSD or HS. Excessive salivation usually started before the dosing of an animal was completed. Many animals developed a conditioned response and would begin to salivate when removed from their run in preparation for dosing. The salivation generally subsided by the afternoon observation period. Excessive thirst, hunched posture, and increased salivation were observed with low

incidence in animals treated with D-70, and sporadically in those treated with RL. Decreased appetite was distributed equally among the groups. Swelling of the injected leg was observed only in HSD- or HS-treated animals, and resolved within 48 hours in each case.

Gastrointestinal signs included vomiting (40 of 60), soft stool (37 of 60), and diarrhea (2 of 60) (Table 4). Vomiting was observed in all HSD- (18 of 18) and HS-treated (18 of 18) animals, and usually occurred within 1 hour after dosing. Vomiting occurred with a lower incidence in D-70-treated animals (4 of 18), and was not observed at all in animals treated with RL. The frequency of vomiting decreased over the 14-day study period. The frequency of vomiting for week 2 was approximately half of that observed in the first week. Soft stool was observed with relatively equal distribution among the groups and study days. Diarrhea was observed only in 2 HSD-treated animals, and resolved within 48 hours in each case.

Respiratory signs included increased respiratory depth (15 of 60), panting (12 of 60), increased respiratory rate (3 of 60), and congestion (3 of 60) (Table 4). Increased respiratory depth was observed with greatest incidence in animals treated with HSD (7 of 18), followed by those treated with HS (5 of 18) and D-70 (3 of 18). Panting was most prevalent in animals receiving D-70 (6 of 18), followed by HS (4 of 18), and HSD (2 of 18). Increased respiratory rate and congestion were sporadically

observed among the groups. No respiratory signs were observed in animals receiving RL.

Serum Chemistry

Serum Na, K, Cl concentrations were similar in all groups throughout the experimental period (Table 5). Because there were no difference among groups at all doses infused, data is only presented from animals that received the MTD.

Serum enzyme activity following daily infusion of HSD at the MTD for 14 days responded similarly as in the acute studies except that the elevation in ala and asp AT and AP continued throughout the experimental period (Fig 3&4). Since the highest elevations in serum enzyme concentrations were observed in the dogs infused daily at the MTD, only those data are depicted.

In dogs infused daily at the MTD of HSD or its individual components, HS or D-70, no significant differences in serum creatinine or BUN were observed among the groups throughout the 14 day experimental period (Table 6).

DISCUSSION

In the present study, 6 hours following infusion of a single bolus of the MTD of 20 ml/kg of HSD to euvolemic dogs, serum Na and K concentrations were significantly higher and lower, respectively, than pre-infusion levels although peak concentrations were not determined. At 24 hours, Na and K concentrations had returned to normal. This is in agreement with previous studies showing a peak rise in serum Na within minutes after an infusion of HSD or hypertonic saline, then a slow return of serum Na towards normal levels. [1,3,4] This return to baseline occurs more rapidly in animals allowed free access to water following HSD infusion, and under these conditions Na concentrations are normal by 24 hours after infusion. [4] Since animals in the present study were allowed free access to water, it is not surprising that serum Na concentrations are normal 24 hours after HSD infusion.

In addition, the decrease in serum K observed in dogs 6 hours following a single MTD of HSD was transient and was not clinically significant. However, since hematocrit and serum protein concentrations were not affected 6 hours after HSD infusion,[25] this effect on serum K cannot be readily explained by simple hemodilution and may be related to increased renal excretion of Na and K following HSD infusion. However, urine was not collected in this study to confirm this hypothesis.

From the discussion above it is not surprising that serum Na and K concentrations did not appear to be affected by daily dosing, since blood samplings were made about 24 hours after each subsequent dose. In addition, serum creatinine and BUN concentrations support the idea that even daily dosing of HSD at the MTD does not compromise renal function.

In the present study, infusion of a single dose of HSD or its constituents at the MTD (5-times the proposed therapeutic dose), [5,6] induced some degree of behavioral abnormalities, although these were transient in nature. These effects were more pronounced in the HSD and HS groups than the other groups, but these effects were more uniformly distributed among all the groups in the daily dosing subacute toxicity studies. A behavioral profile similar to HSD and its constituents in the present study was observed in our laboratory under the same infusion protocols in rabbits. [26,27] In rabbits, however, HS infusion at the MTD induced the greatest behavioral disturbances and was lethal within 12 min of infusion in 6 of 20 rabbits. [26] Although symptoms resembling seizures were not observed in these studies, induction of seizures is a major clinical concern in response to a hypernatremia. In the present study, a cause-effect relationship between hypernatremia and behavioral disturbances can only be inferred by the higher incidence of abnormalities in the HSD and HS groups, but blood samples were not collected early enough to determine the peak serum Na concentrations. In recent preliminary studies in sheep infused with 4 ml/kg of HS solutions

ranging from 3% to 25%, plasma Na concentrations exhibited a dose-dependent increase. In the 25% HS group, plasma Na concentrations exceeded 200 mEq/l within 2-3 min following infusion, and then quickly fell to more clinically accepted levels after 1 hr. [28] Since the total Na load in the present studies is greater than in this sheep study, it is quite possible that the transient behavioral disturbances observed are related to a hypernatremia. However, this would require verification. Nevertheless, the other symptoms are consistent with volume and fluid overload previously observed in toxicity studies with earlier clinical dextran preparations.[21,26]

In the single dose studies at the MTD, serum enzyme levels, as a clinical profile for liver damage, showed only transient changes. Similar results were observed in the rabbit studies.[26] A number of earlier studies have shown that infused dextran can accumulate in the liver, [19,20,29-32] and we have recently shown that following infusion of 4 ml/kg HSD, less than 10% remains in the liver after 4 days.[33] Despite this storage in liver and other tissues, dextran does not seem to be associated with any adverse effects on liver function, [34] and it is completely catabolized over time.[29]

Daily infusion of HSD or its components induced a more sustained increase in serum enzyme concentrations, although the levels did begin to decrease after the third day. A similar profile was also observed in our earlier study in rabbits.[26] Although the daily dosing studies were not extended to

investigate the reversibility of the enzyme response once the infusions stopped, both gross and microscopic examination of liver and other major organs failed to detect signs of tissue damage (data not shown). In addition, it is important to note that in the multiple infusion studies, serum enzyme levels never exceeded the values observed in the acute toxicity studies. These data would imply that the effects of multiple infusions of HSD or its components are not additive.

In conclusion, since the proposed therapeutic dose of HSD is a single infusion of 4 ml/kg, the present data suggest that its use should be associated with minimal toxicity.

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Table 1

CLINICAL OBSERVATIONS FOLLOWING A SINGLE BOLUS INFUSION (20 ml/kg) IN DOGS[1]

	Solution Infused			
Observation	RL	HSD	HS	D70
Behavioral	0/4	4/4	4/4	2/4
Gastrointestinal	0/4	4/4	3/4	1/4

[1]Data represent number of animals observed/number of animals per group.

Table 2
Serum Electrolyes in Dogs Infused with 20ml/kg of The Test Solutions

		Day 0	0.25	1	3	7	14
Na (mEq/l)	RL	153±1	152±2	152±2	155±2	150±5	153±2
	HSD	152±2	157±1*	152±1	152±3	151±1	145±2
	D-70	152±2	151±2	152±2	151±1	149±1	150±1
	HS	152±2	156±1*	151±2	151±1	138±5	149±3
K (mEq/l)	RL	5.3±0.1	5.1±0.2	4.8±0.2	5.0±0.2	4.8±0.2	5.0±0.2
	HSD	5.2±0.1	4.1±0.1*	4.7±0.1	5.0±0.2	5.2±0.2	4.8±0.2
	D-70	5.1±0.2	5.1±0.1	4.8±0.2	4.8±0.2	4.8±0.2	4.8±0.2
	HS	5.1±0.2	4.4±0.1*	4.6±0.1	4.8±0.1	4.3±0.2*	4.8±0.2
Cl (mEq/l)	RL	111±1	115±1	111±1	116±1	114±2	113±2
	HSD	110±1	116±2	112±1	113±2	112±1	109±4
	D-70	113±1	114±1	113±1	113±1	114±1	113±1
	HS	112±1	116±1	111±1	114±1	107±3	112±2

[1]Data expressed as mean ± S.E. from 4 dogs/group.
*$p<0.05$ from day 0 value and corresponding RL and D-70 groups.

Table 3
Effect of a single MTD of HSD or its Components on Blood Urea Nitrogen and Serum Creatinine Concentations[1]

		Day						
		0	0.25	1	2	3	7	14
BUN (mg/dl)	RL	19.3±2.4	20.0±1.6	15.8±1.6	16.8±0.9	18.7±2.4	18.4±2.2	14.4±1.8
	HSD	16.3±1.2	12.4±2.3*	12.9±1.6	16.4±2.5	14.8±0.7	17.4±3.3	13.8±0.9
	D-70	17.2±2.6	20.0±1.6	15.3±1.3	20.3±2.6	17.2±1.1	16.8±2.7	15.6±1.4
	HS	16.6±1.6	15.0±0.7	14.1±1.2	17.3±1.9	16.5±1.1	17.8±1.1	13.8±0.8
Creatinine (mg/dl)	RL	0.73±0.10	0.68±0.06	0.73±0.02	0.70±0.07	0.75±0.06	0.75±0.06	0.80±0.07
	HSD	0.68±0.05	0.55±0.03*	0.75±0.06	0.70±0.08	0.68±0.08	0.68±0.05	0.73±0.06
	D-70	0.70±0.04	0.65±0.03	0.80±0.04	0.70±0.04	0.73±0.05	0.73±0.02	0.80±0.04
	HS	0.65±0.03	0.50±0.0*	0.75±0.03	0.75±0.03	0.73±0.02	0.60±0.04	0.78±0.02*

[1]Data expressed as mean ±S.E. for 4 animals/group.
* P<0.05 from day 0 basline values.

Table 4
CLINICAL OBSERVATIONS FOLLOWING DAILY INFUSIONS IN DOGS[1]

Observation	Dose(ml/kg)	RL[2]	Solution Infused HSD	HS	D70
Behavioral	12		6/6	6/6	4/6
	16		6/6	6/6	3/6
	20	2/6	5/6	6/6	4/6
Gastrointestinal	12		6/6	6/6	1/6
	16		6/6	6/6	2/6
	20	4/6	6/6	6/6	6/6
General	12		5/6	5/6	2/6
	16		6/6	6/6	2/6
	20	1/6	6/6	6/6	1/6
Other	12		1/6	2/6	2/6
	16		0/6	1/6	2/6
Panting Nasal	20	0/6	1/6	1/6	2/6

[1]Data presented as number of observations/number of animals per group.
[2]RL was only administered at 20 ml/kg.

Table 5
Serum Electrolytes in Dogs Infused Daily with the MTD of Each Test Solution[1]

		Day 0	1	3	7	14
Na (mEq/l)	RL	115±2	153±1	152±1	115±1	152±1
	HSD	153±1	156±2	151±1	152±1	151±1
	D-70	154±1	155±2	148±4	152±1	150±1
	HS	153±1	153±3	153±1	154±2	153±1
K (mEq/l)	RL					
	HSD	4.9±0.2	4.7±0.1	4.8±0.1	4.4±0.1	4.6±0.1
	D-70	4.9±0.1	4.7±0.1	4.6±0.2	4.4±0.1	4.6±0.1
	HS					
Cl (mEq/l)	RL	116±1	116±1	115±1	117±1	116±1
	HSD	114±1	120±2	116±1	117±1	118±1
	D-70	116±1	118±1	117±1	119±1	118±1
	HS	114±1	119±1	114±1	115±2	113±1

[1]Data expressed as mean ± S.E. of 6 dogs/group.

Table 6

Effects of Daily Infusions of the MTD of HSD and its Components on Blood Urea Nitrogen and Serum Creatinine[1]

		Day				
		0	1	3	7	14
BUN (mg/dl)	RL	17.5±2.5	15.4±0.7	17.8±1.5	19.0±1.4	17.6±1.0
	HSD	16.1±0.4	13.2±0.7	16.8±1.1	16.8±0.8	16.4±0.8
	D-70	16.0±0.8	16.4±1.4	16.8±1.4	16.9±2.4	16.4±0.9
	HS	17.9±2.2	15.6±1.7	17.9±1.2	17.5±1.5	19.3±1.0
Creatinine (mg/dl)	RL	0.68±0.02	0.60±0.09	0.70±0.04	0.72±0.04	0.82±0.03
	HSD	0.75±0.03	0.72±0.05	0.68±0.03	0.72±0.05	0.70±0.06
	D-70	0.72±0.02	0.68±0.04	0.67±0.03	0.68±0.03	0.68±0.05
	HS	0.77±0.02	0.75±0.02	0.72±0.02	0.73±0.05	0.83±0.06

[1]Data expressed as mean ± S.E. from 6 animals/group.

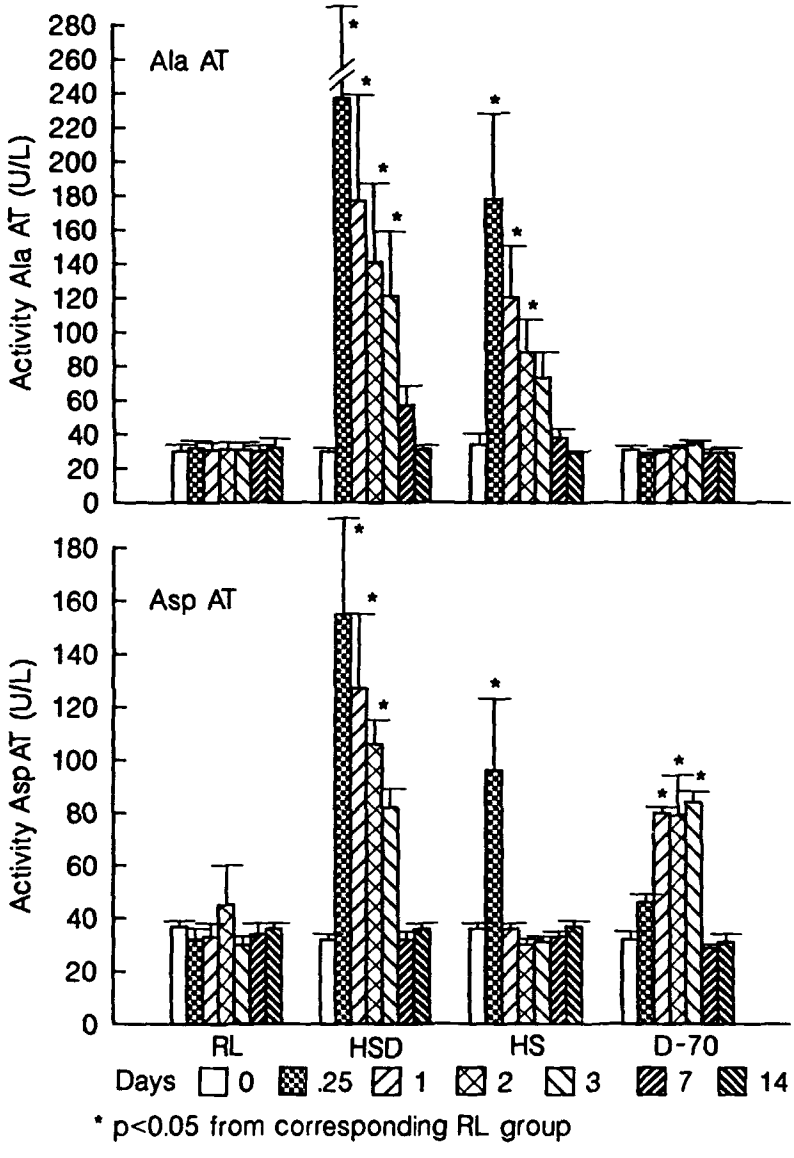
Legends to Figures

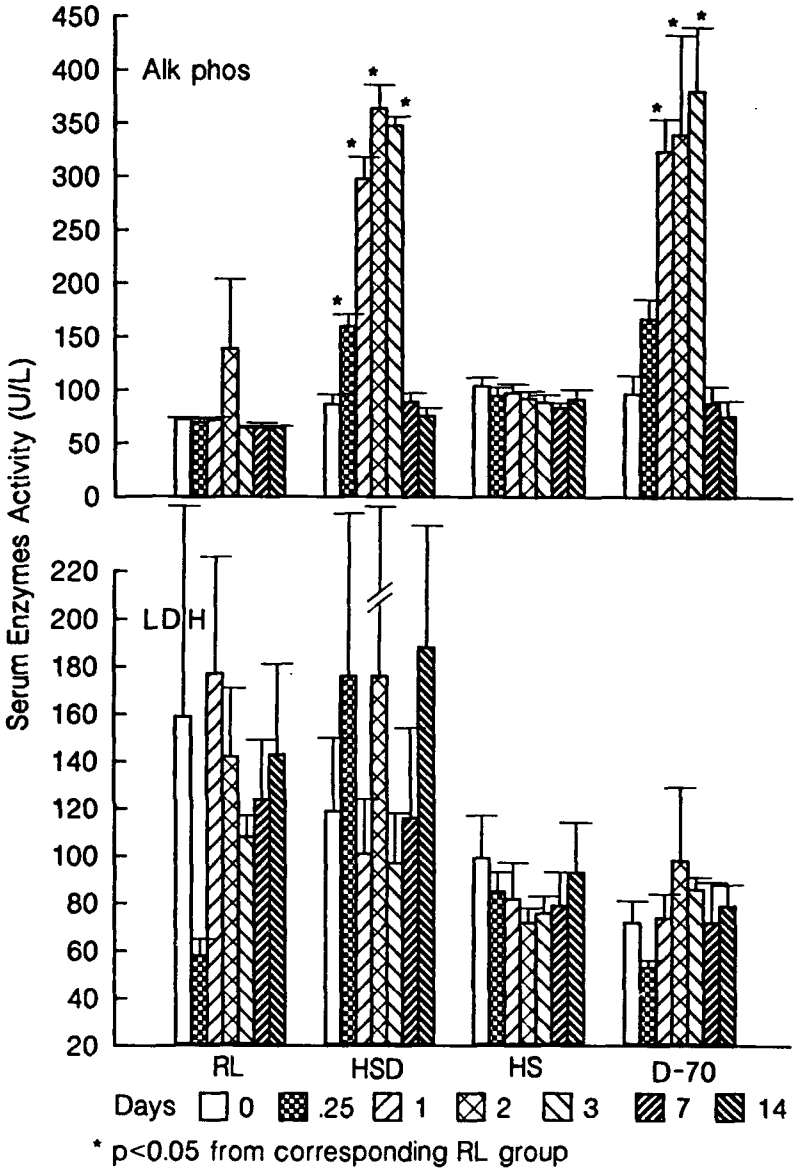
Fig. 1. Alanine and Aspartate Aminotransferase activity in serum from dogs infused with a single dose of 20 ml/kg (MTD) of HSD or its components. Data expressed as mean±S.E. of 4 dogs/group. *p<0.05 from baseline and RL controls.

Fig. 2. Alkaline phosphatase and lactate dehydrogenase activity in serum from dogs infused with the MTD of HSD or its components. Data expressed as mean±S.E. of 4 dogs/group. *p<0.05 from baseline and RL controls.

Fig. 3. Alanine and Aspartate Aminotransferase activity in serum from dogs infused daily for 14 days with the MTD of HSD or its components. Data expressed as mean±S.E. of 6 dogs/group. *p<0.05 from baseline and RL controls.

Fig. 4. Alkaline phosphatase and lactate dehydrogenase activity in serum from dogs infused daily for 14 days with the MTD of HSD or its components. Data expressed as mean±S.E. of 6 dogs/group. *p<0.05 from baseline and RL controls.


Ala AT
Activity Ala AT (U/L)
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
Asp AT
Activity Asp AT (U/L)
180
160
140
120
100
80
60
40
20
0
RL
HSD
HS
D-70
Days 0 .25 1 2 3 7 14
* p<0.05 from corresponding RL group


Alk phos
LDH
Serum Enzymes Activity (U/L)
450
400
350
300
250
200
150
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50
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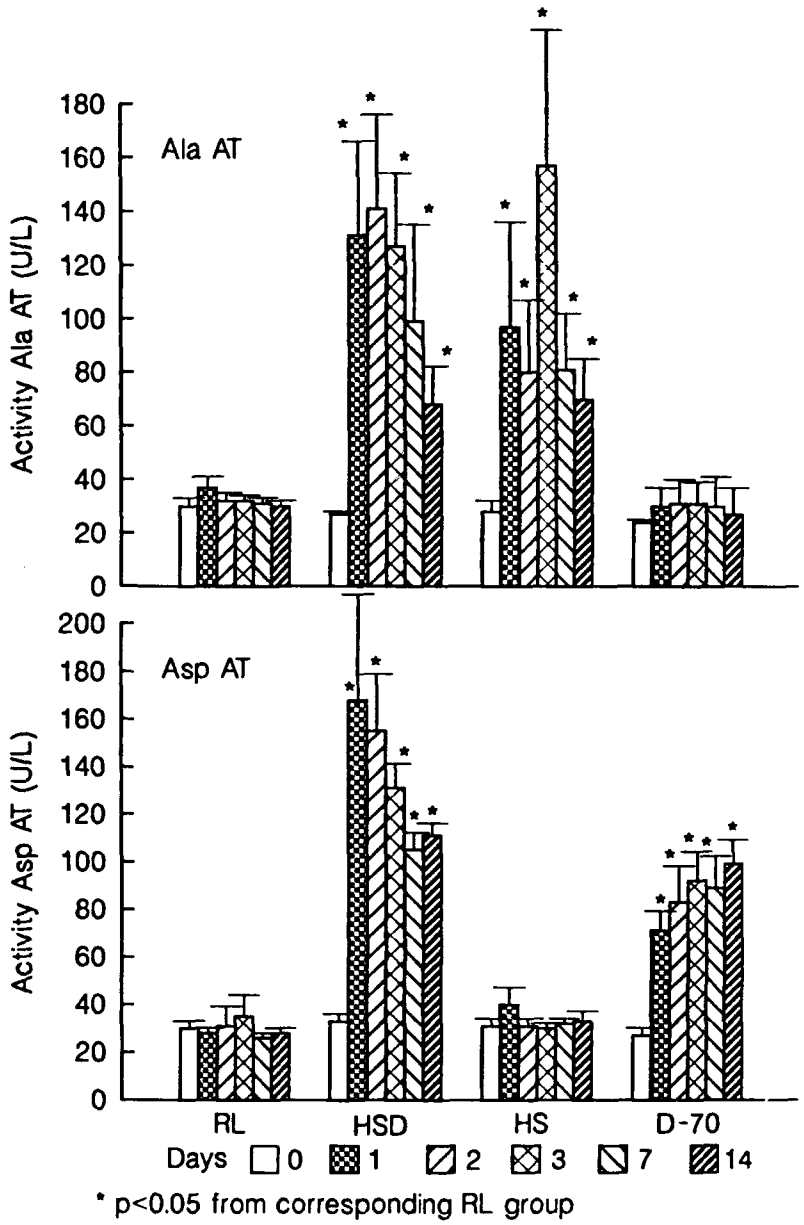
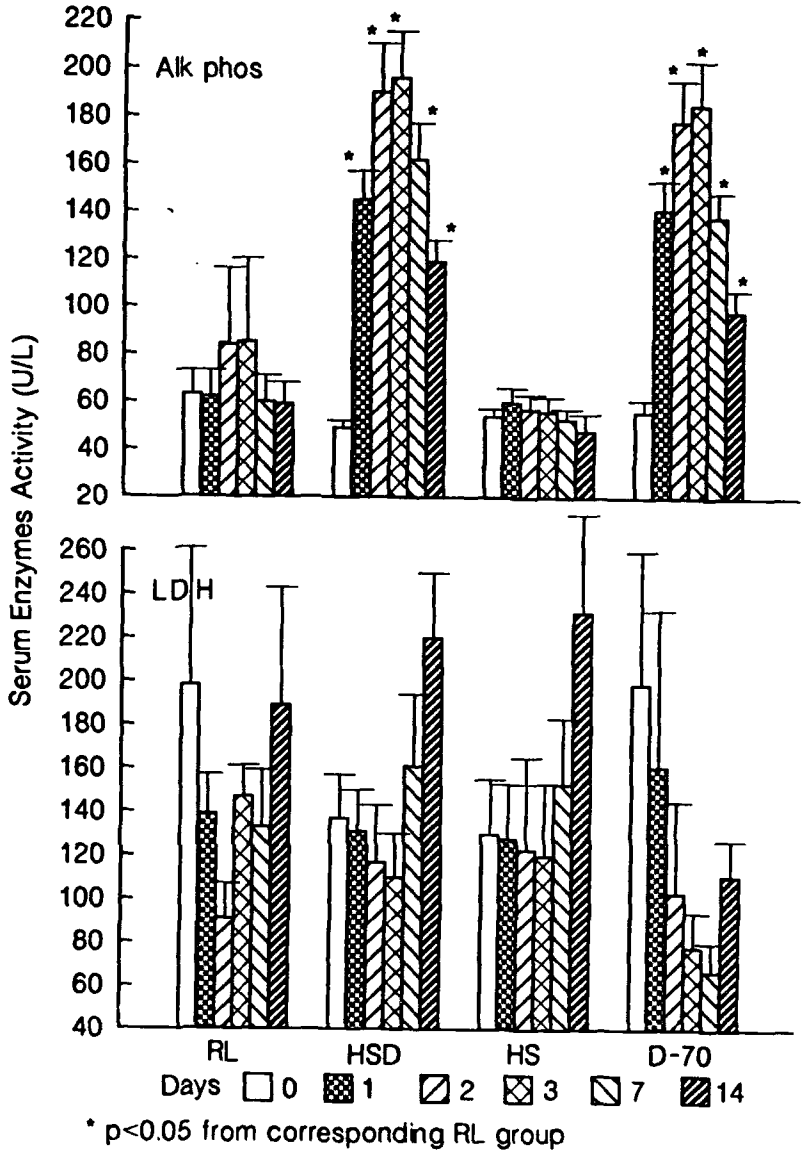
160
140
120
100
80
60
40
20
RL
HSD
HS
D-70
Days 0 .25 1 2 3 7 14
* p<0.05 from corresponding RL group


Ala AT
Activity Ala AT (U/L)
180
160
140
120
100
80
60
40
20
0
Asp AT
Activity Asp AT (U/L)
200
180
160
140
120
100
80
60
40
20
0
RL
HSD
HS
D-70
Days 0 1 2 3 7 14
* p<0.05 from corresponding RL group


Alk phos
LDH
Serum Enzymes Activity (U/L)
220
200
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RL
HSD
HS
D-70
Days 0 1 2 3 7 14
* p<0.05 from corresponding RL group